Old Target, New Approach:
Developing Pterin-like Small Molecules as Inhibitors of the Bacterial Folate Pathway

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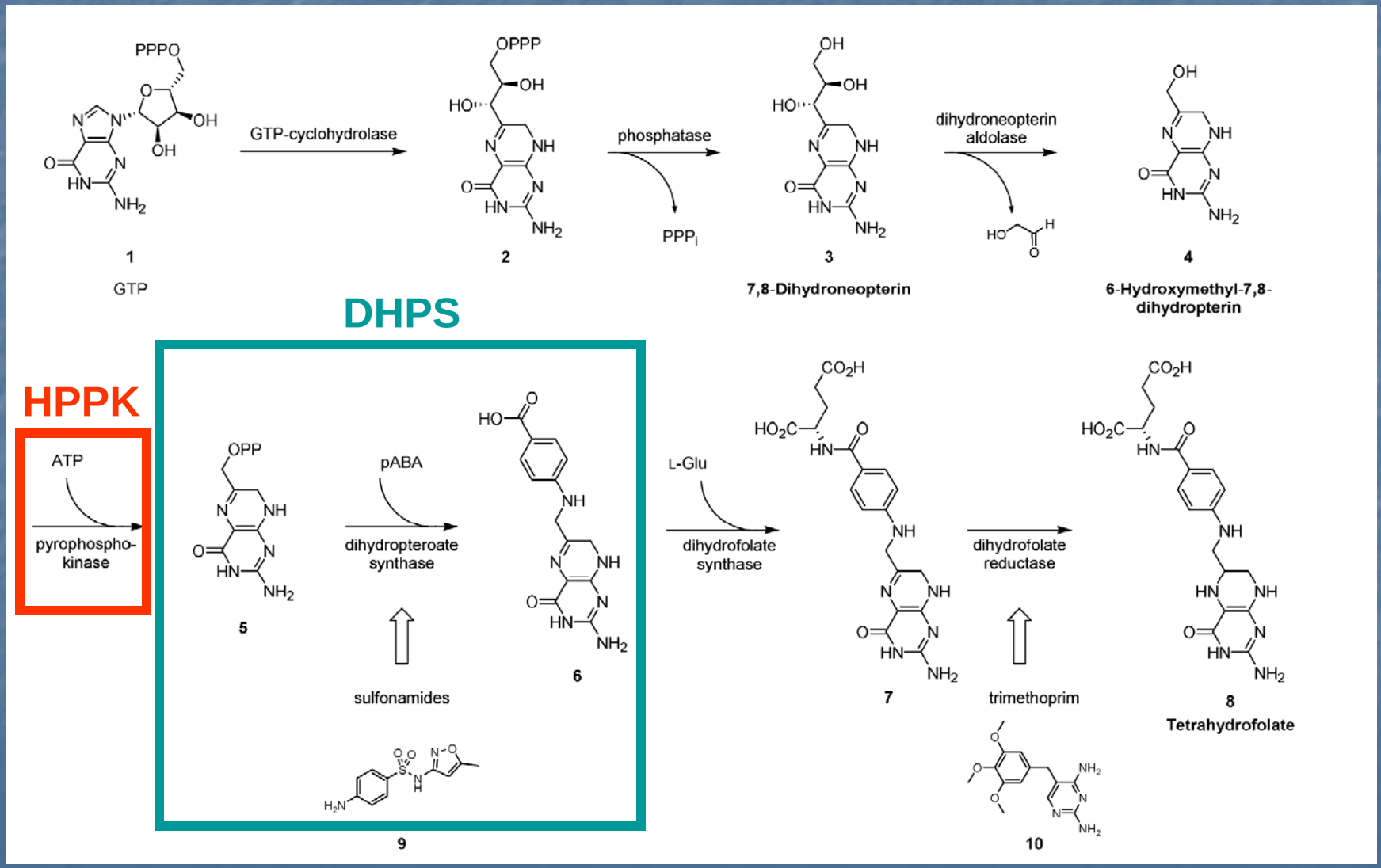
Folate Synthesis: Established Target Revisited

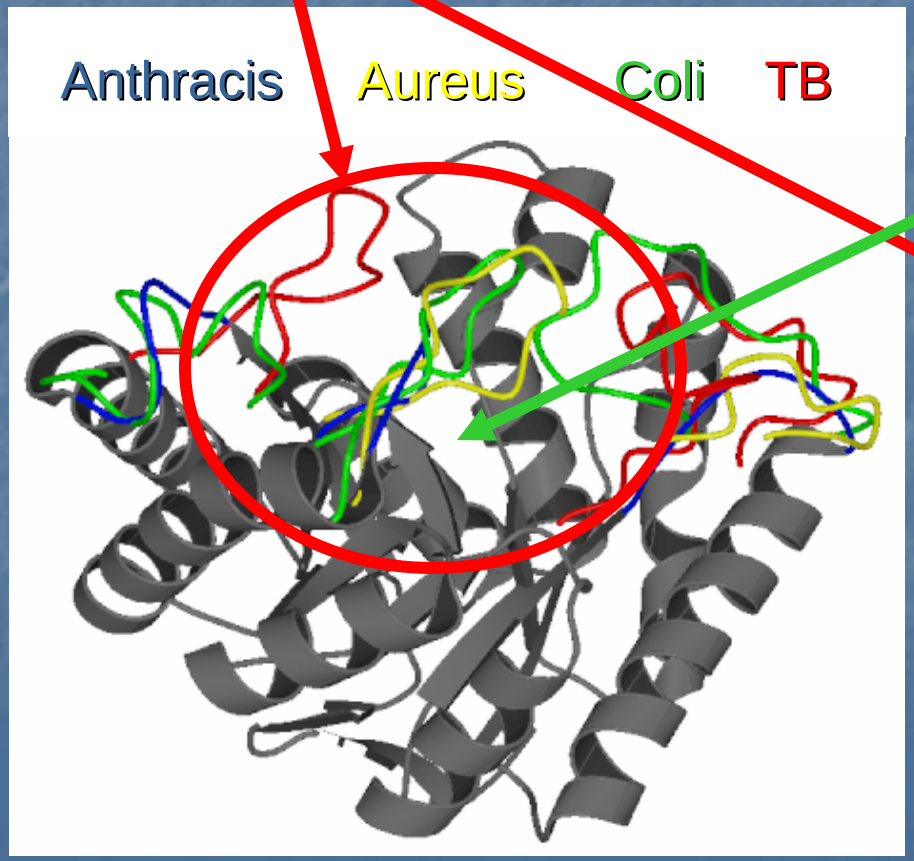


Why Target DHPS?

- DHPS is a proven drug target.
  - Sulfonamides marketed since 1930's
  - Historically susceptible pathogens include many gram-positive, gram-negative, fungal, and protozoal species
  - Drugs of choice for several disease states (UTI's, PCP, SSTI's)
  - Sulfonamides, sulfones, and DHFR combos are still considered first line agents for many bacterial pathogens (*Pneumocyctis sp*.)
- Crystal structures now known for four bacterial pathogens *(E. coli, S. aureus, M. tuberculosis, B. anthracis).*
- *p*ABA (sulfonamide) and pterin binding sites have now be visualized and key binding interactions modelled for structure guided drug discovery.
- New role for DHPS inhibitors in emerging diseases such as MRSA and VRSA.

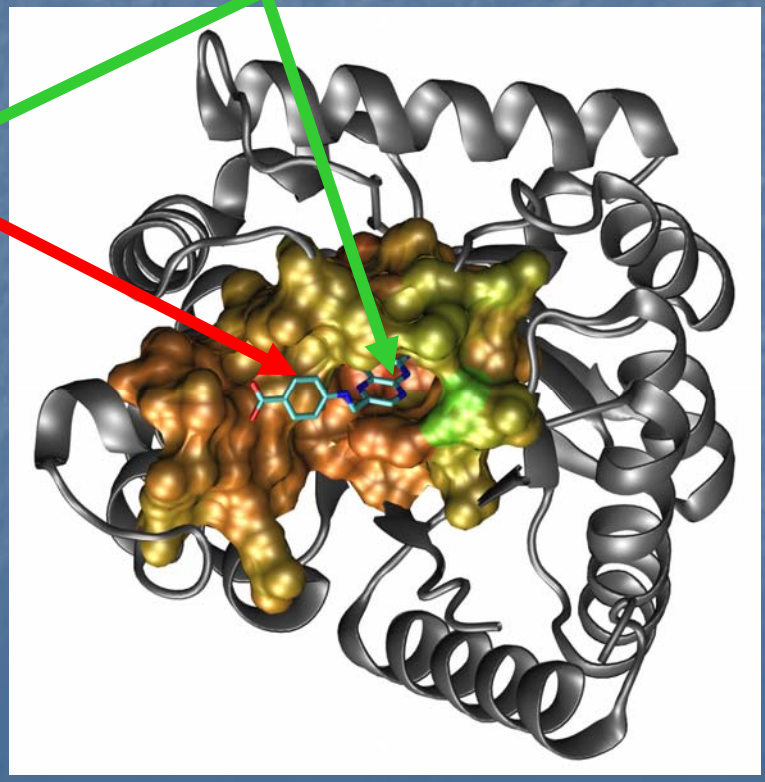
Central Idea of the Project:
Identify Inhibitors that Target the Pterin Pocket

1. *p*ABA (and sulfur drugs) bind at a surface region surrounded by flexible loop regions. Easy to accommodate resistance mutations



3. Identify small molecule inhibitors that engage the pterin pocket.

2. Pterin-PP binds in a conserved, deep pocket. Difficult to accommodate mutations.



Scientific Scope of the Project

Two Parts:

Part 1 Target development

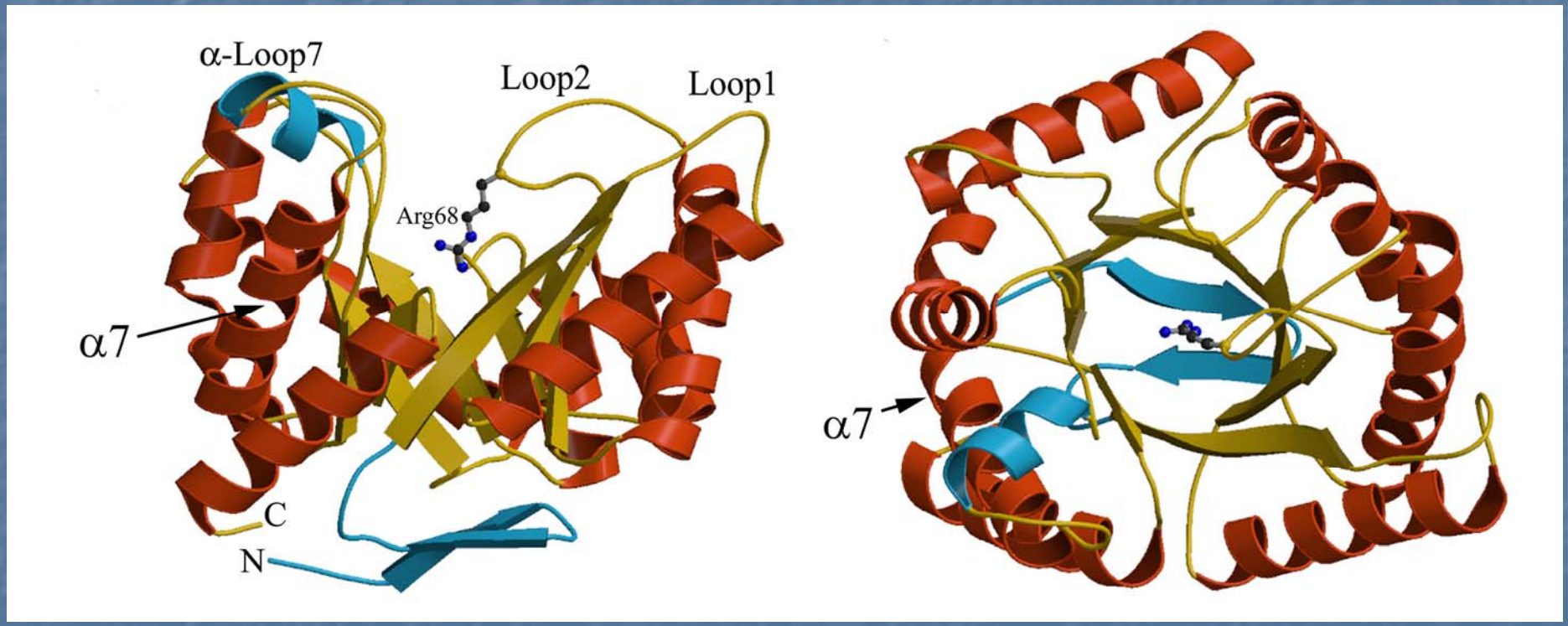
Part 2 Development of novel inhibitors

Scientific Scope of the Project

Part 1 Target development

- Clone, express, purify, crystallize DHPS from *B. anthracis, Y. pestis, F. tularemia* and *M. tuberculosis* and determine the structures.
- Determine the structures of DHPS-substrate and -product complexes.
- Probe the catalytic mechanism.
- Understand the structural basis of sulfa-drug resistance.

B. anthracis DHPS Monomer



Studies on the *Francisella tularenis* Enzyme:
Sequence Analysis Reveals a Fused HPPK-DHPS Bifunctional Enzyme

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                1         10         20         30         40         50         60
F.tularensis    MQYIIGIGTNIGFTIENIHLAITALESQQNIRIIRKASLYSSKAVLKEDAPKEWDIRFLNTAVK
E.coli          TVAYIAIGSNLASPLEQVNAALKALGDIPESHILTVSSFYRTPPLGPQDQPD.....YLNAAVA
consensus>50    mvyiIaIGsNiafpiE#!nlAikALediq#ihIirvaSlYrskavlk#DqPdewdir%LNaAVa

                    70         80         90        100        110        120
F.tularensis    ISSSLKPDELLVLLKDIELKIGRDLNAPAWSPRVIDLDILAAEDLILETDKLTIPHKELINRSF
E.coli          LETSLAPEELLNHTQRIELQQGRVRKAERAGPRTLDLDIMLFGNEVINTERLTVPHYDMKNRGF
consensus>50    iesSLaP#ELLvllqdIELqiGRvlnAeaagPRviDLDI$lfe#l!i#T#kLT!PHy#$iNRgF

            130        140        150        160                170        180
F.tularensis    ALAPLLELSKGWHHPKYVEWDLNIRLKELGEIVKLKQ.......TLANTIRMGIVNLSNQSFSD
E.coli          MLWPLFEIAPELVFPDGEMLRQILHTRAFDKLNKWKLFAQGTSLDLSHPHVMGILNVTPDSFSD
consensus>50    mLaPLlEiakelvfPdyvmldliihlkeldeivKlKlfaqgtsldLanpivMGIvNvsn#SFSD

                190        200        210        220        230        240
F.tularensis    GNFDDN..QRKLNLDELIQSGAEIIDIGAESTKPDAKPISIEEEFNKLDEFLEYFKSQLANLIY
E.coli          GGTHNSLIDAVKHANLMINAGATIIDVGGESTRPGAAEVSVEEELQRVIPVVEAIAQR.....F
consensus>50    Gnfd#nli#avlnl#l$I#aGAeIID!GaESTkPdAae!S!EEEl#kvievvEyiaqqlanli%

            250        260        270        280        290        300        310
F.tularensis    KPLVSIDTRKLEVMQKILAKHHDIIWMINDVECNNIEQKAQLIAKYNKKYVIIHNLGITDRNQY
E.coli          EVWISVDTSKPEVIRESAKVGAHII...NDIRSLSEPGALEAAAETGLPVCLMHMQGNPKTMQE
consensus>50    evl!S!DTrKlEViqeilavgadIIwmiND!eclnieqal#liAeynlkvviiHmlGipdrmQy

                320        330        340        350        360        370
F.tularensis    LDK.ENAIDNVCDYIEQKKQILLKHGIAQQNIYFDIGFGFGKKSDTARYLLENIIEIKRRLELK
E.coli          APKYDDVFAEVNRYFIEQIARCEQAGIAKEKLLLDPGFGFGKNLSHNYSLLARLAEF.HHFNLP
consensus>50    ldKy##vid#VndYii#qiqillqaGIAq#nillDiGFGFGKnldhnyyLLeniiEikhhl#Lk

                380        390        400        410        420
F.tularensis    ALVGHSRKPSVLGLAKDSNLATLDRATRELSRKLEKLDIDIIRVHKI................
E.coli          LLVGMSRK.SMIGQLLNVGPSERLSGSLACAVIAAMQGAHIIRVHDVKETVEAMRVVEATLSAK
consensus>50    lLVGmSRKpSviGlll#vnlaellraslelavilemlddidIIRVHd!ketveamrvveatlsak
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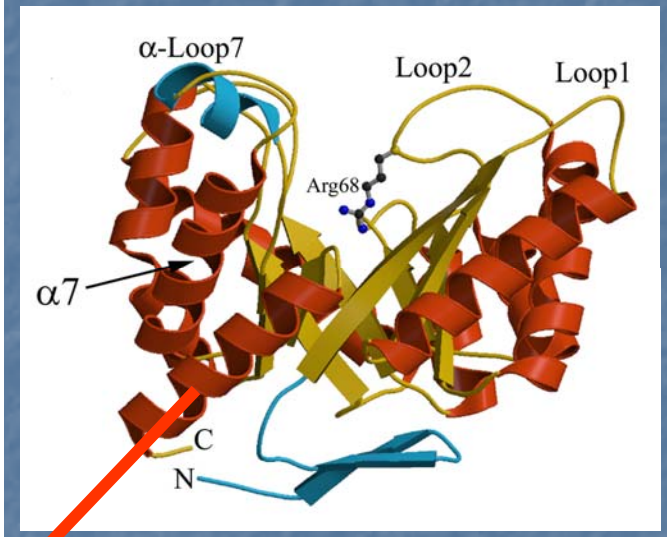
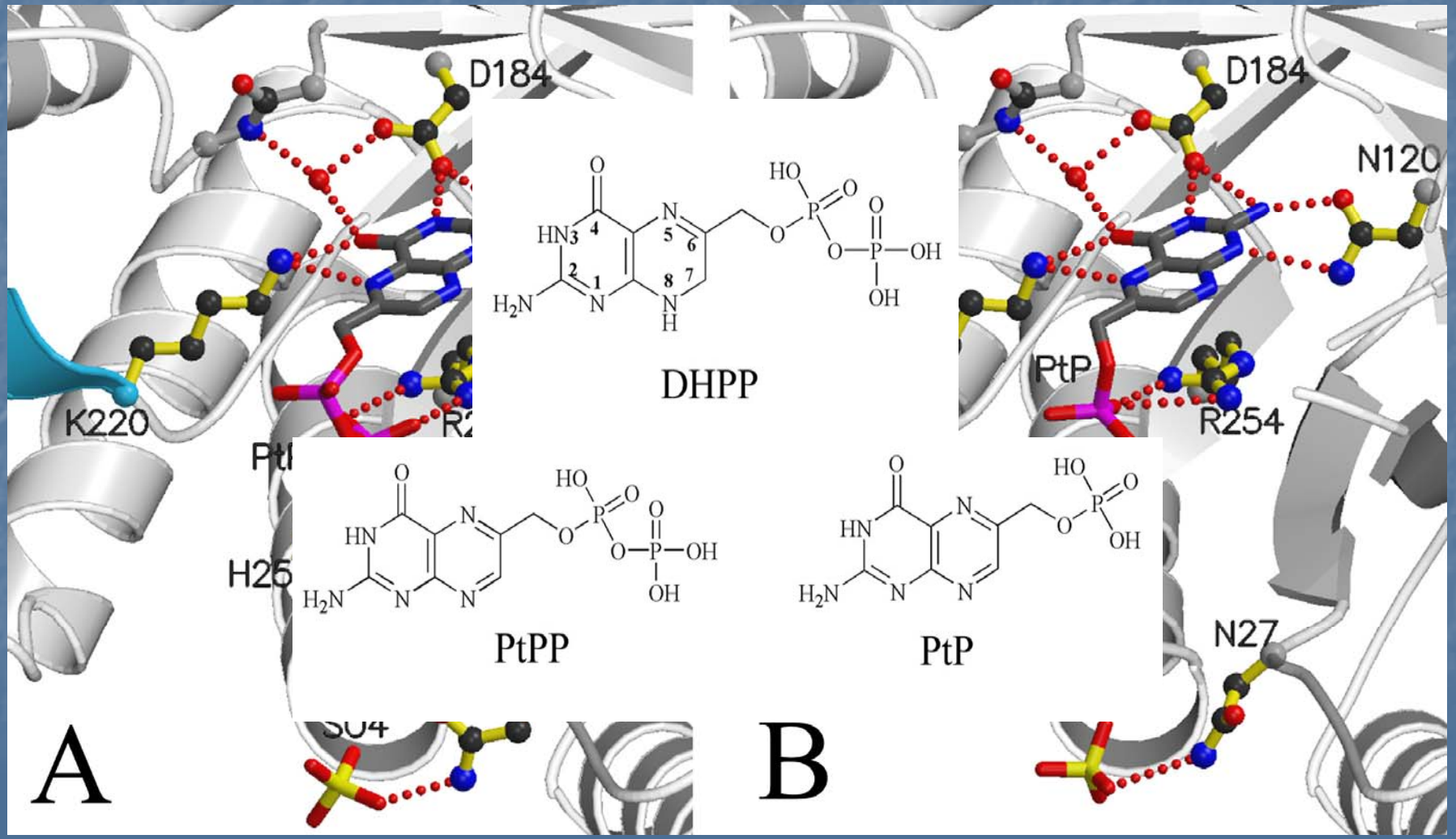
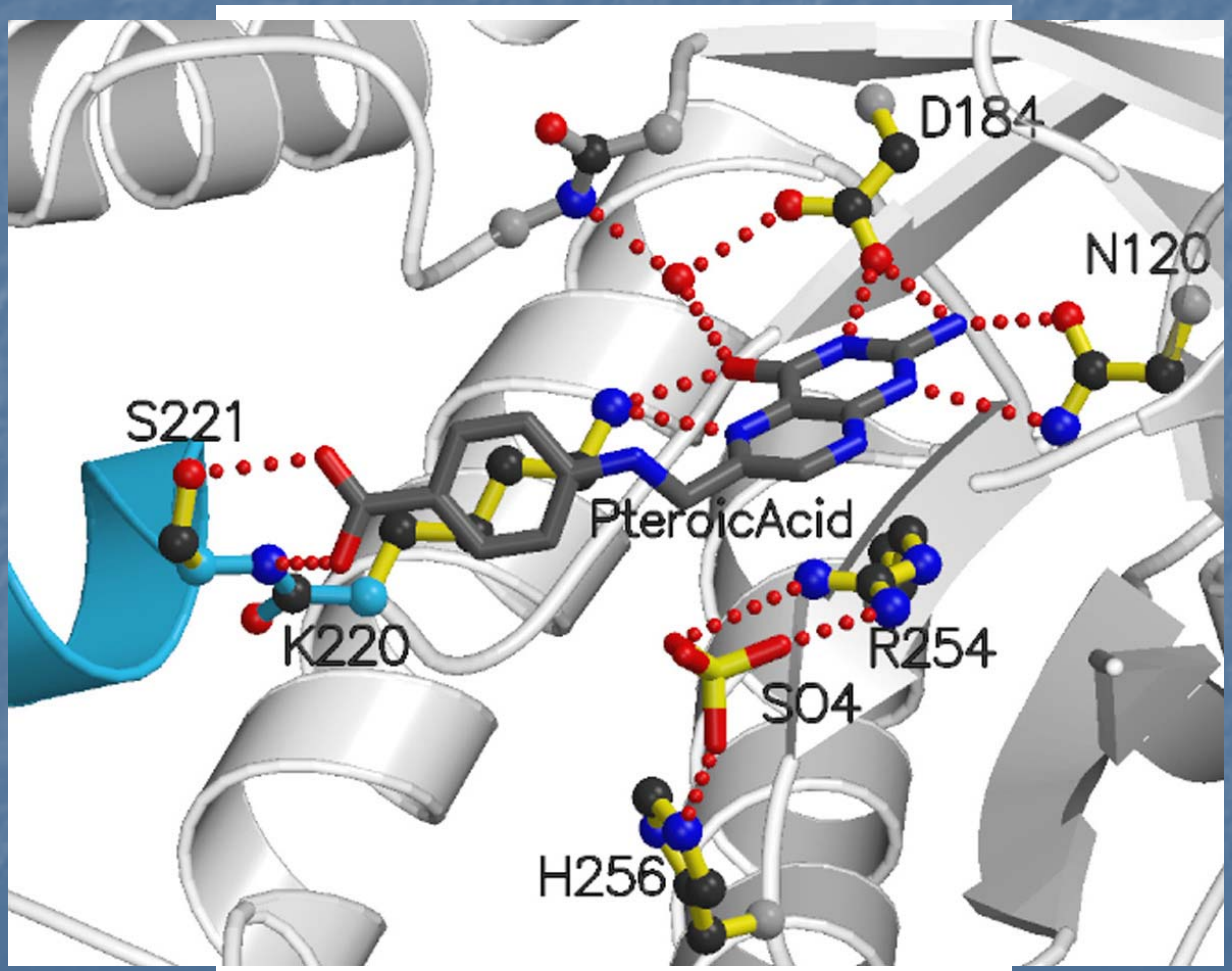


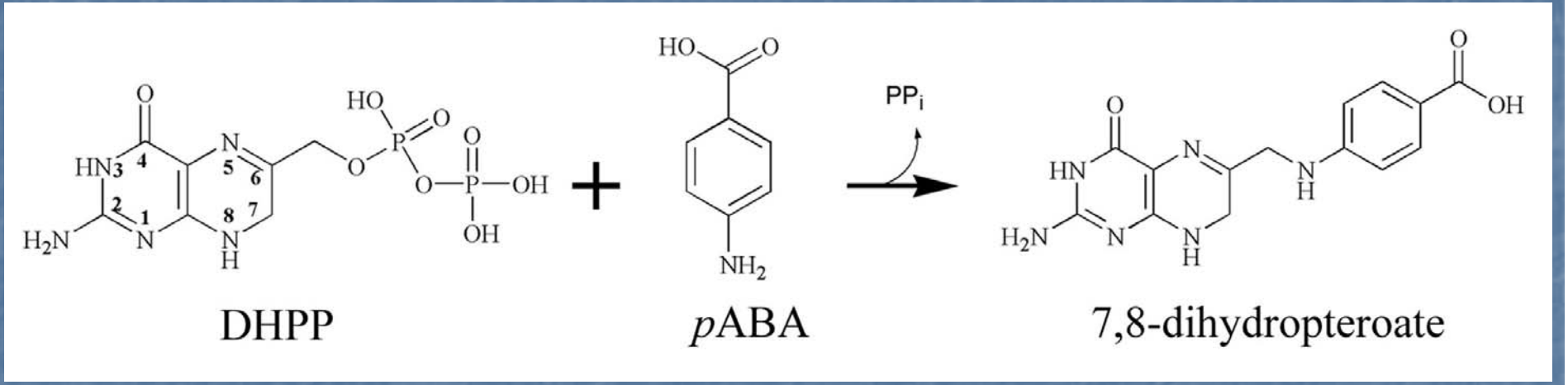
Complexes with PtPP and PtP



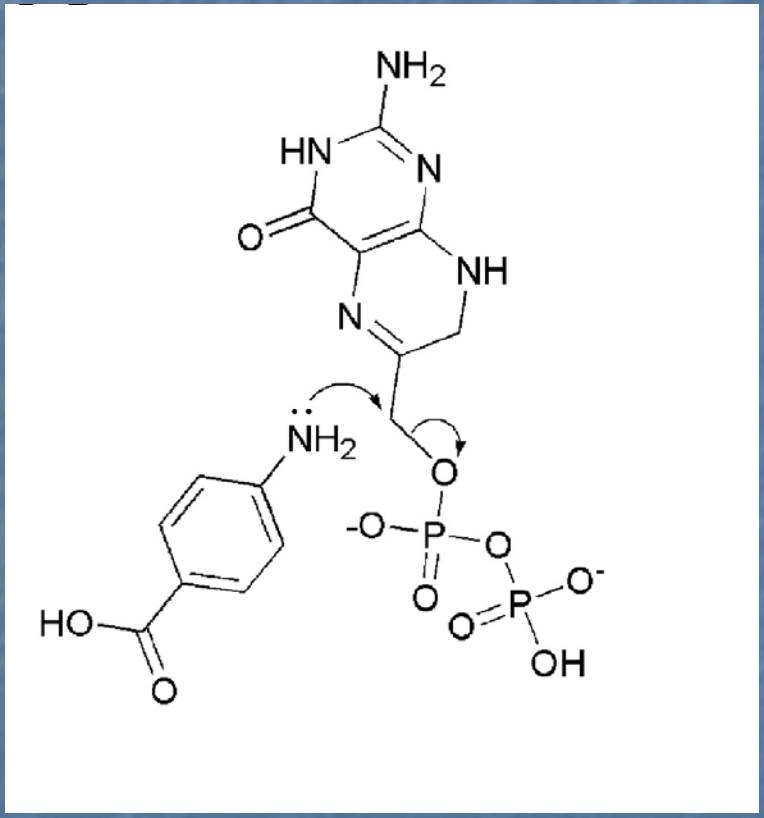
Complex with Pteroic Acid: Product Analog



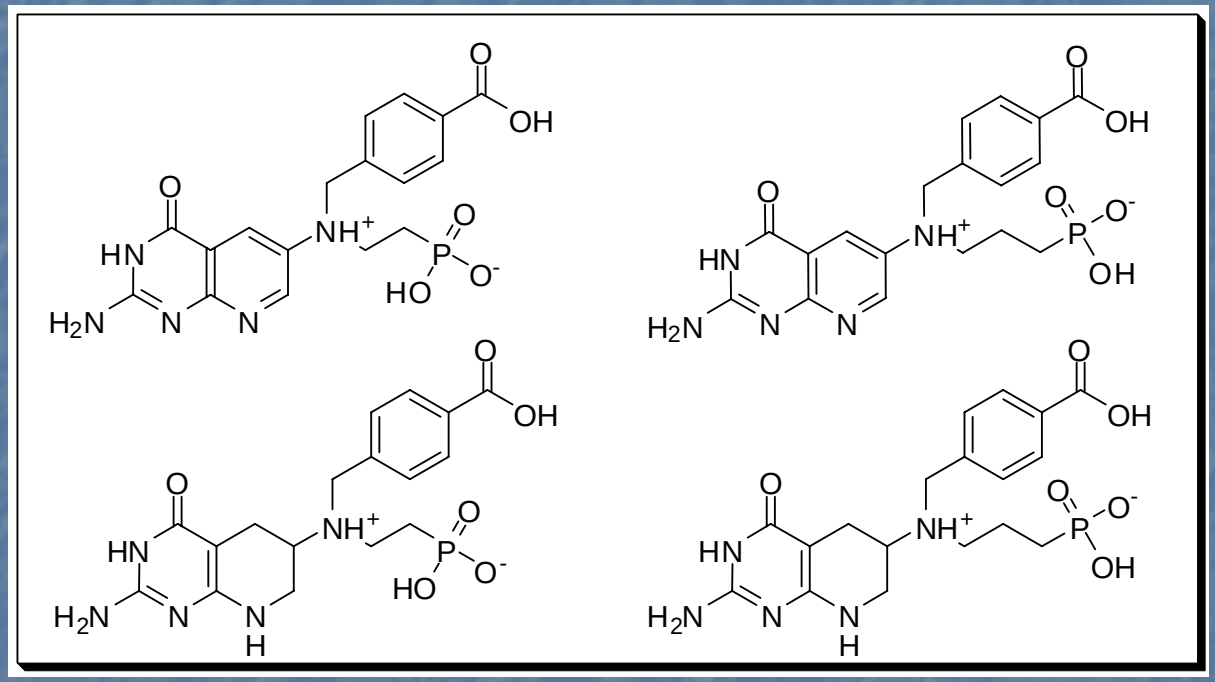
DHPS Reaction



Enzyme Mechanism – SN2?

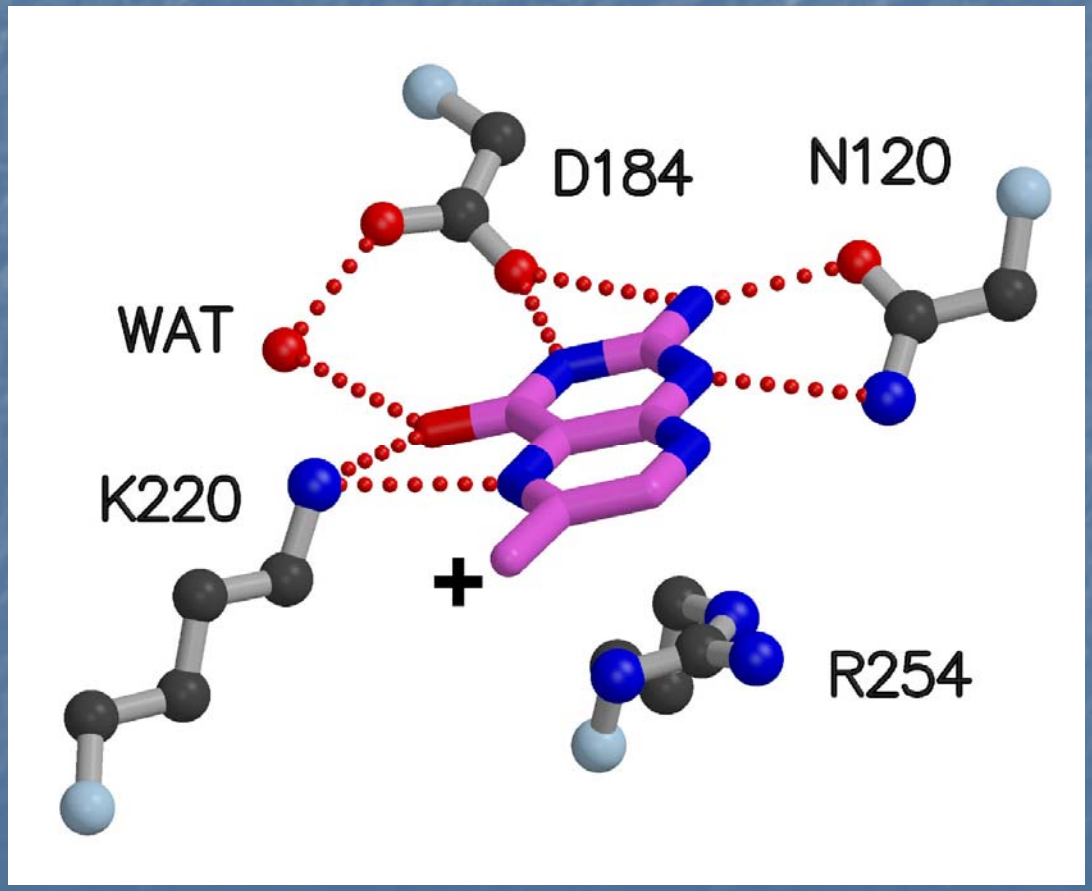


Synthesized DHPS Transition State Analogues

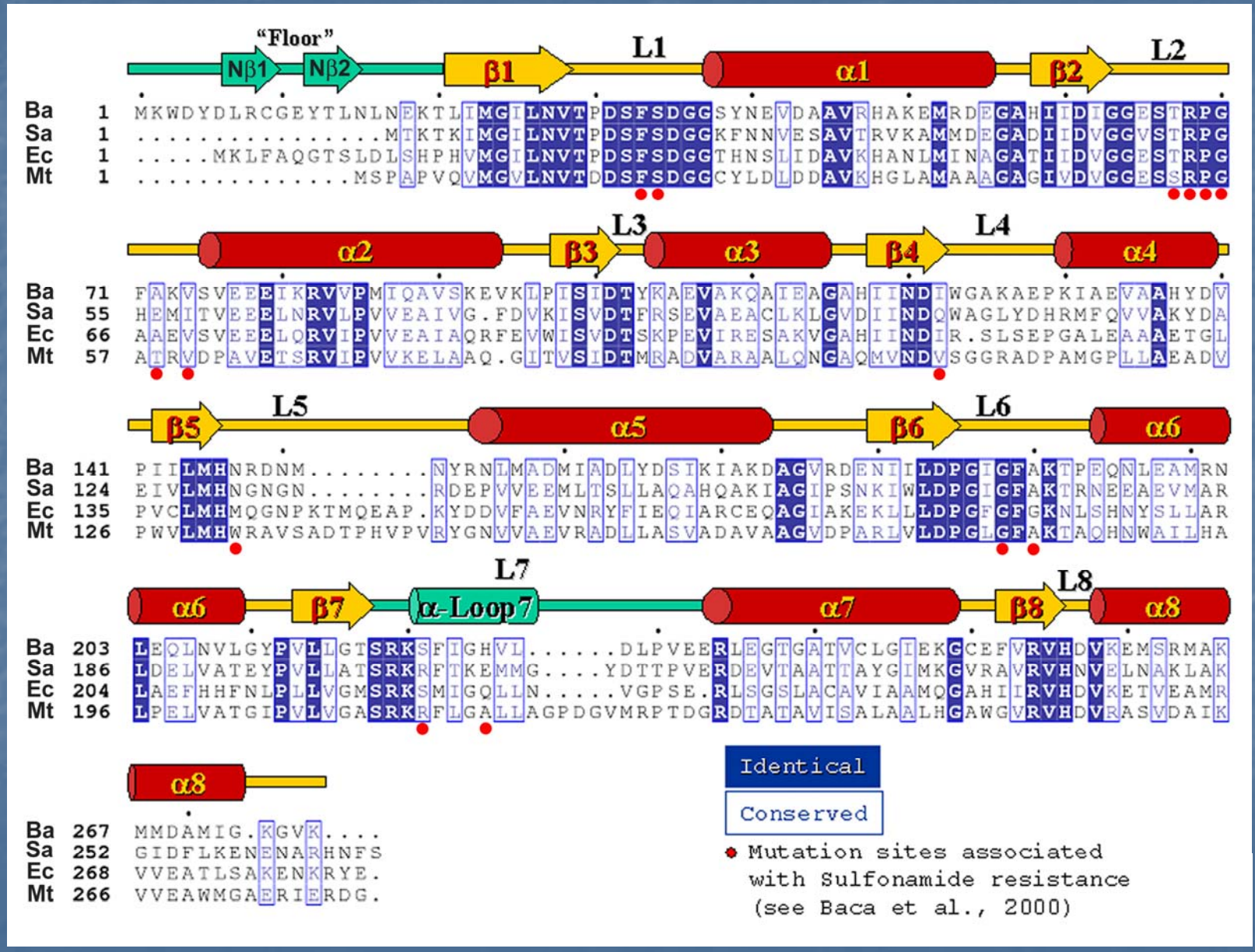


Placement of the amine at the 6’-position on the ring and formation of a tertiary amine-type functionality was designed to stabilize the proposed positive charge of the transition state

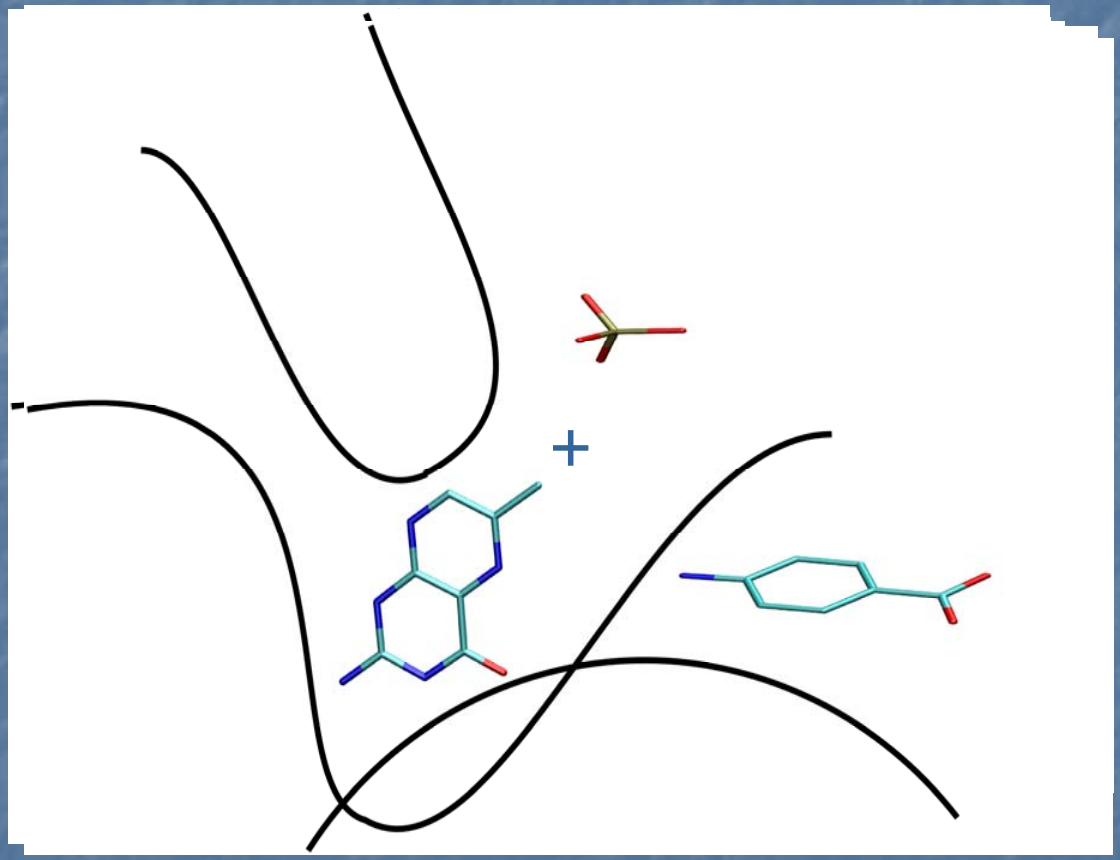
Enzyme Mechanism – SN1?



DHPS Sequence Alignment



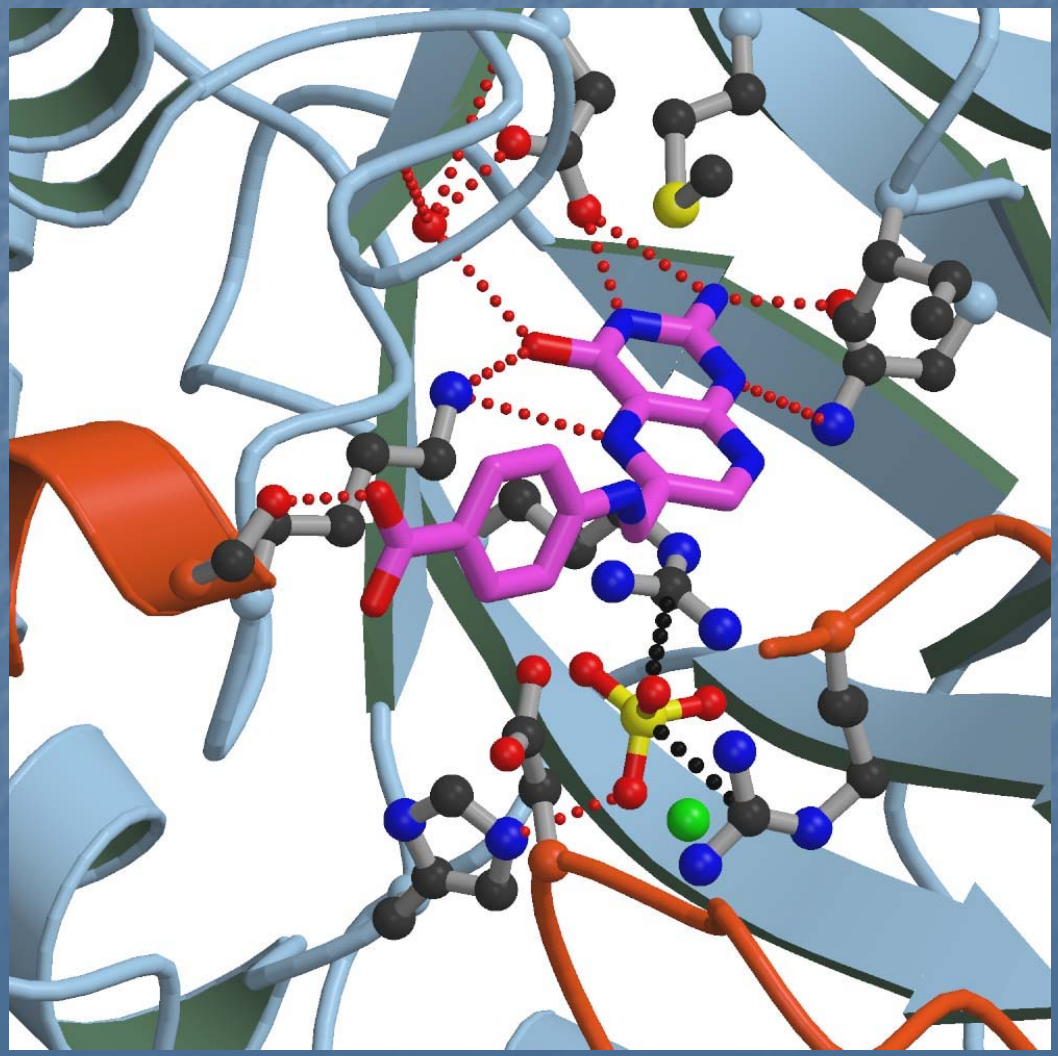
Enzyme Mechanism – Pseudo SN2?



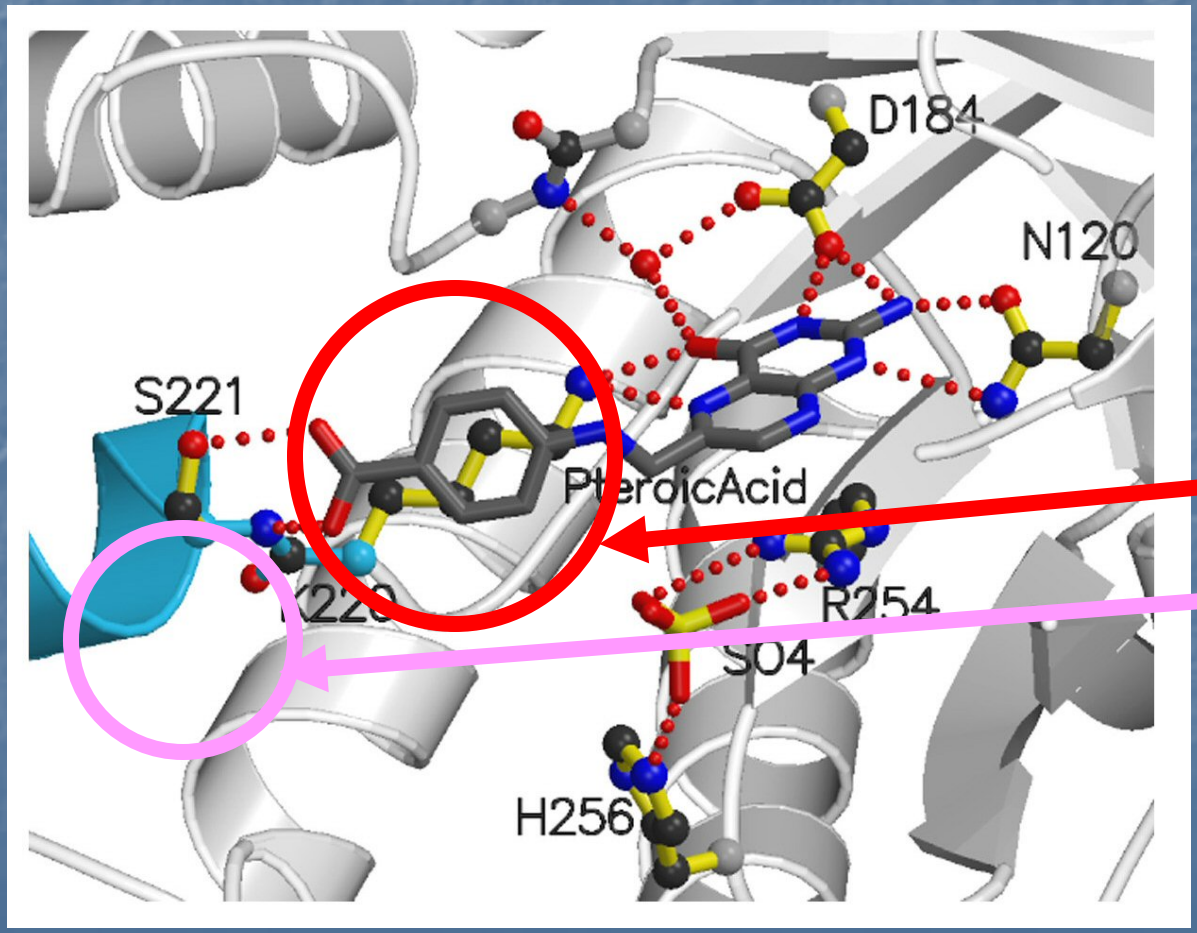
•***1999*** Vinnecombe, et al, demonstrate that the target for sulfonamide inhibition (of *S. pneumoniae*) is the enzyme-DHPP binary complex, rather than the apoprotein form of the enzyme

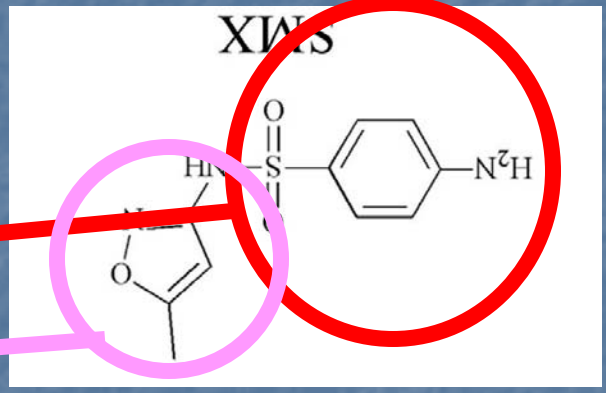
Building up the DHPS Active Site

Combining 3 anthracis structures and 1 from TB, one can gain insights into the full active site and the roles of conserved loops 1 and 2.



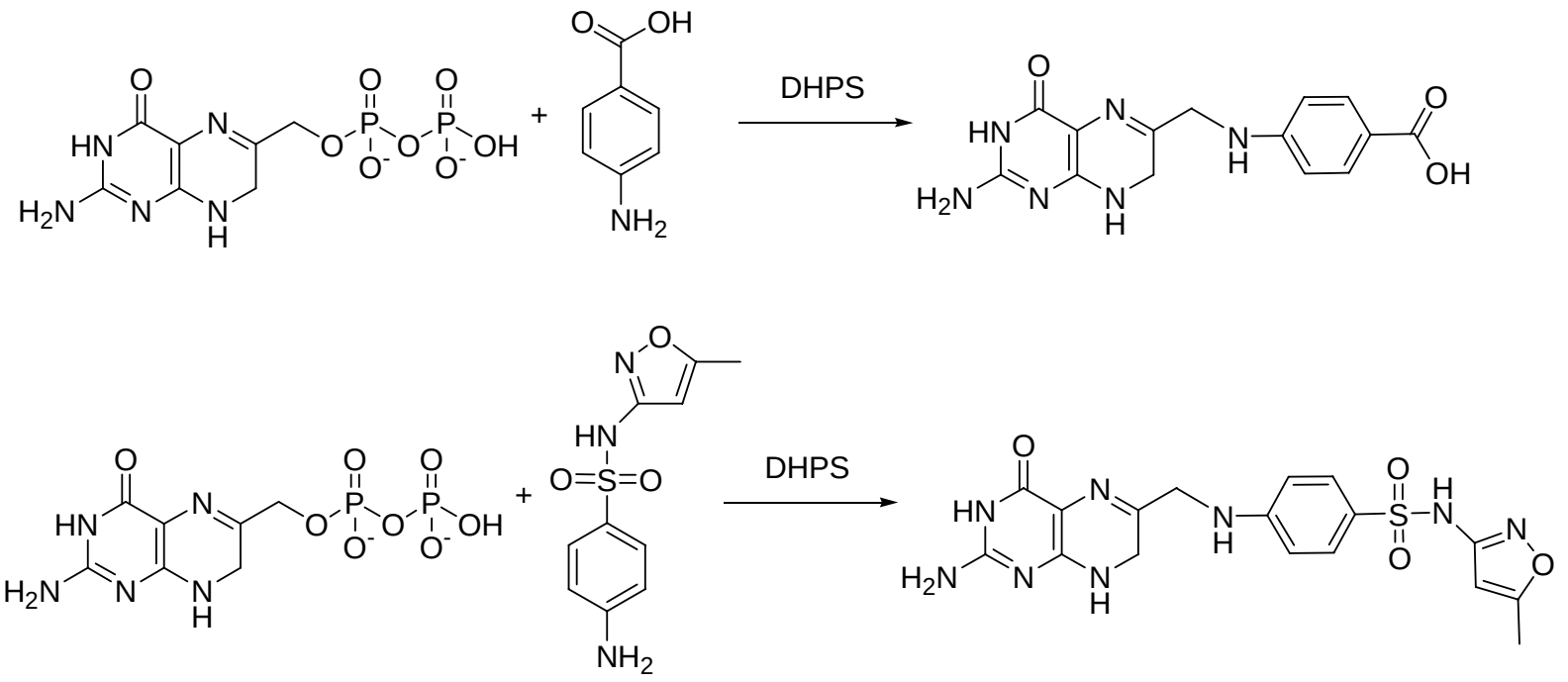
Sulfonamide resistance – Insights from the product analog structure





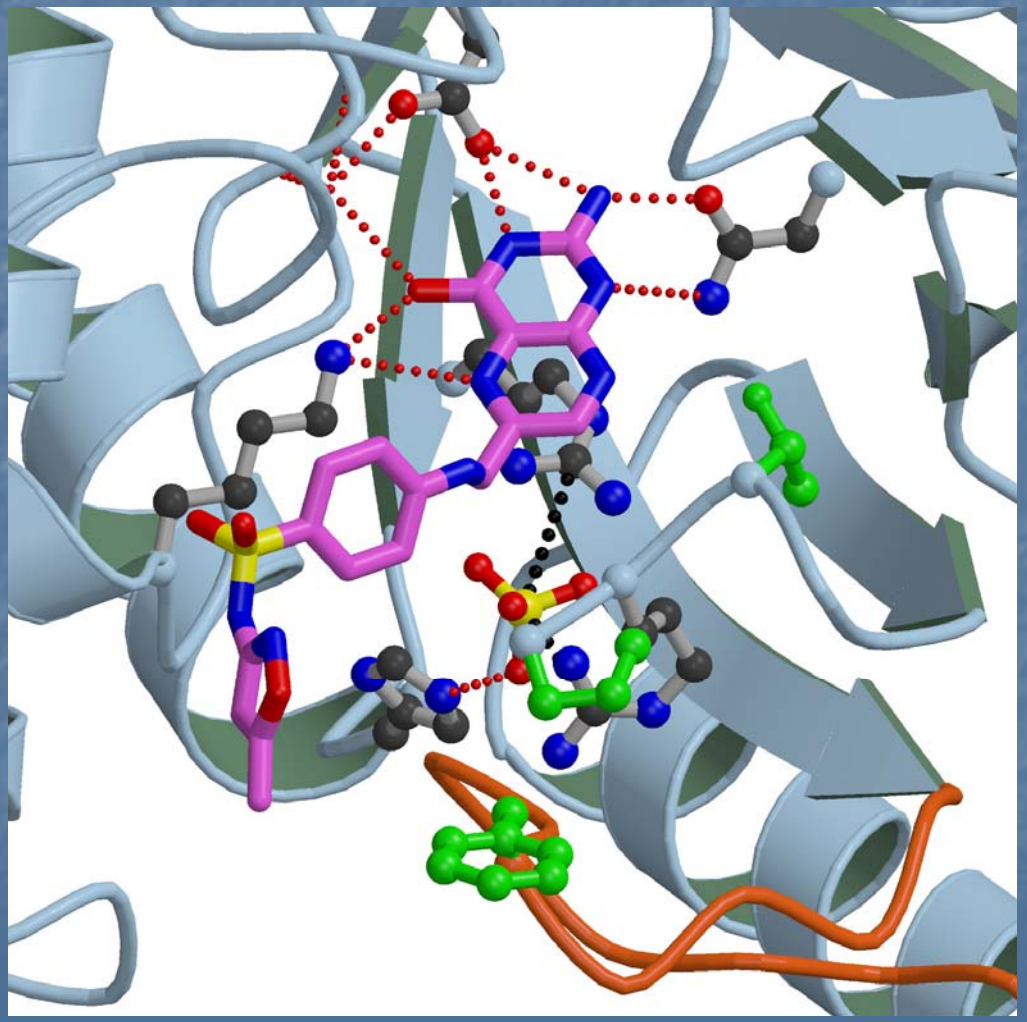
Using Dead-End Products to Understand Sulfa-drug Resistance in DHPS

There is good evidence that at least some of the DHPS enzymes can link the sulfa-drug to the pre-bound pterin-PP substrate. We synthesized this adduct for structural studies.



Inhibitor or just bi-product?

Dead-end Sulfamethoxazole Product Analog Bound to DHPS

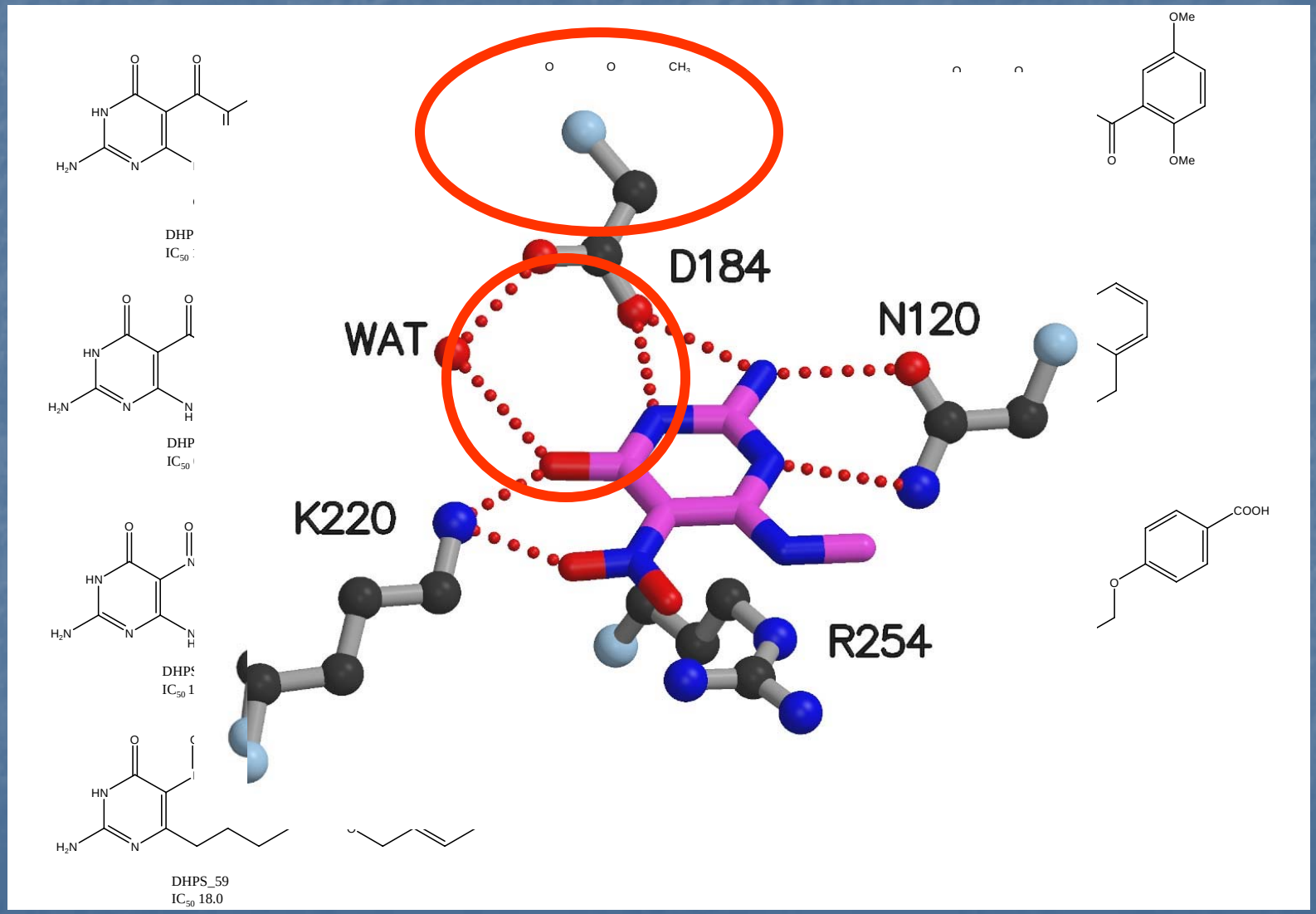


Scientific Scope of the Project

Part 2 Development of novel inhibitors

- Virtual screen new small molecule structures to identify novel scaffolds.
- Develop a suitable enzyme assay.
- Synthesize and screen hit optimization libraries.
- Perform microbiological assessment of inhibitors against the target organisms.

Starting Point 1:
Early Burroughs-Wellcome Studies



Starting Point 2: Virtual screen of *B. anthracis* DHPS

- Pharmacophore search to prefilter library.
- Validation of docking protocol vs *B. anthracis* DHPS.
- Dock, procure and test top 2%.

Pharmacophore search of *B. anthracis* DHPS

- ZINC: 26 databases of commercially available compounds
  - 5 million compounds (multiple tautomeric and protonation states).
  - Pre-filtered for drug-likeness.
- Methods
  - UNITY flex search against active site surface and 5 macro constraints (1 donor atoms, 4 acceptor atoms)
  - 2 Partial Match Dialogs
  - Pharmacophore utilizes 9229 crystal structure
  - Rule of 3 criteria: MW <350, max. rotatable bond 5

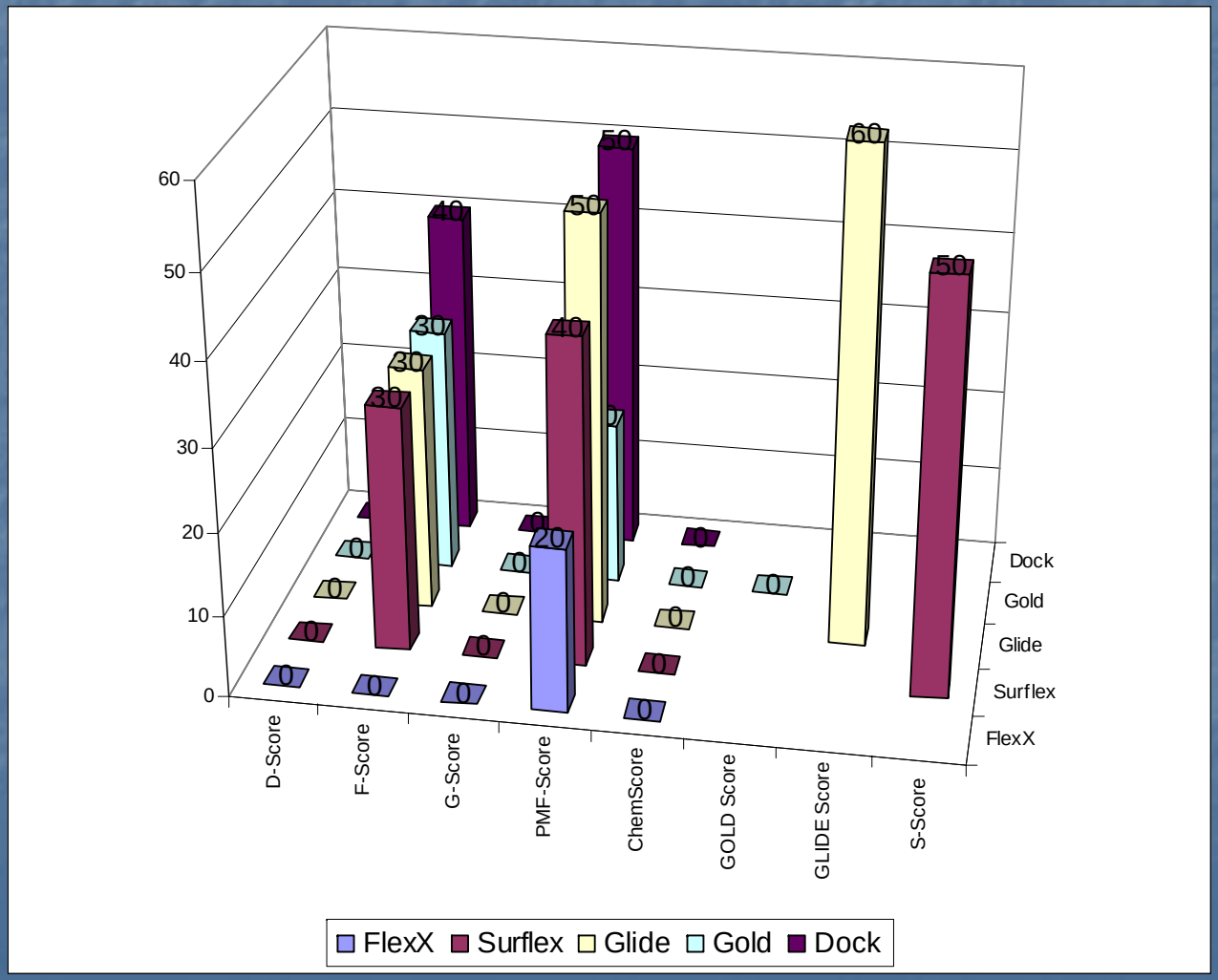
Pharmacophore Prefilter Hits

Peakdale:	24	ACBEurochem:	19
Key Organics:	48	GPCR:	0
ComGenex:	0	TimTec:	318
PubChem:	349	Otava:	78
Life Chemicals:	28	NCI:	1339
Sigma-Aldrich:	207	Asinex:	168
LeadScreen:	1	Interchim:	116
Maybridge:	87	Enamine:	171
LeadQuest:	1	Ambinter:	550
ChemDiv:	286	Chembridge:	147
Heterocycles:	54	Pharmeks:	96
Nanosyn:	132	Specs:	332
RyanScientific:	235	IBScreen:	307

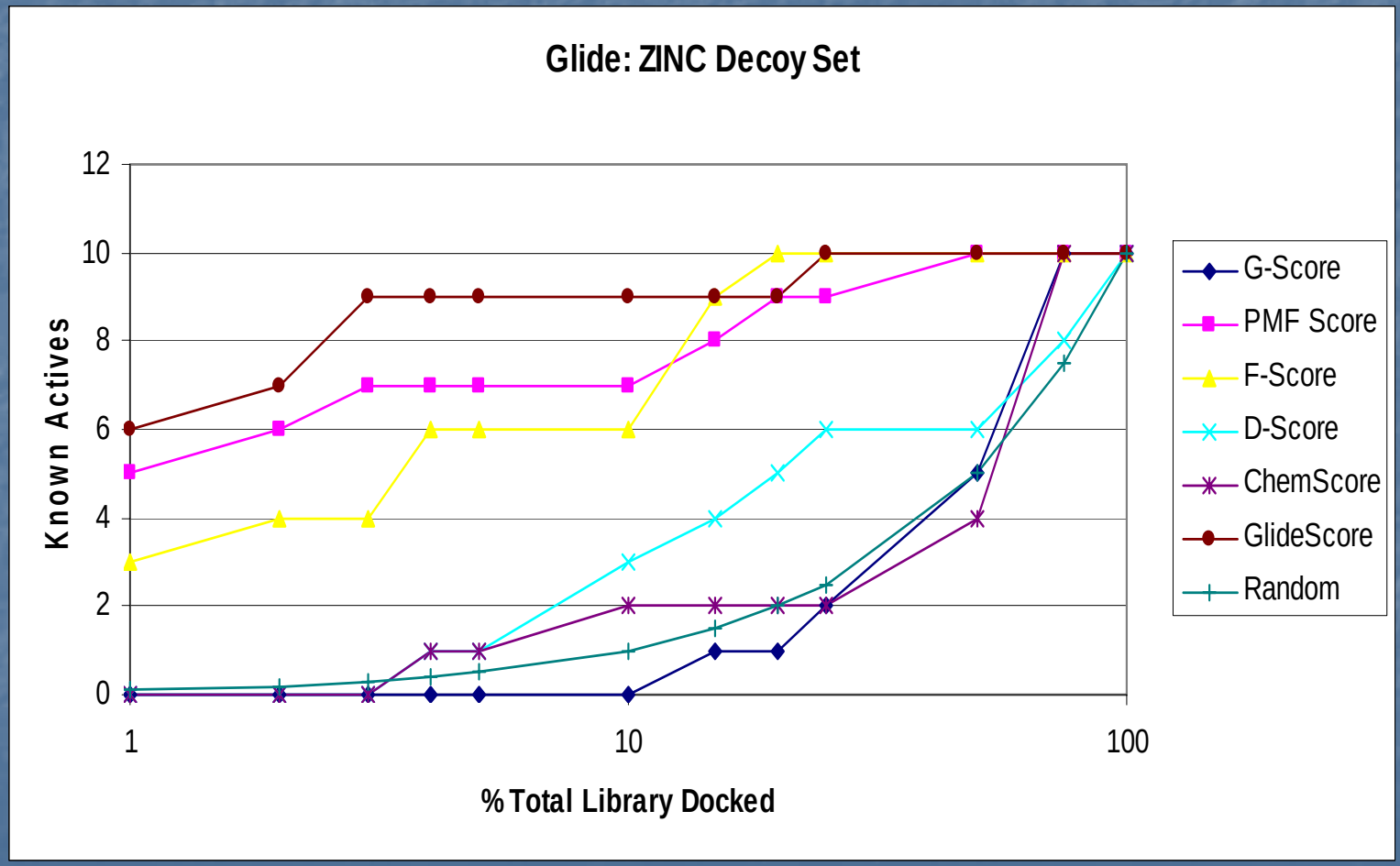
TOTAL: 5093

Unique Total: 3104

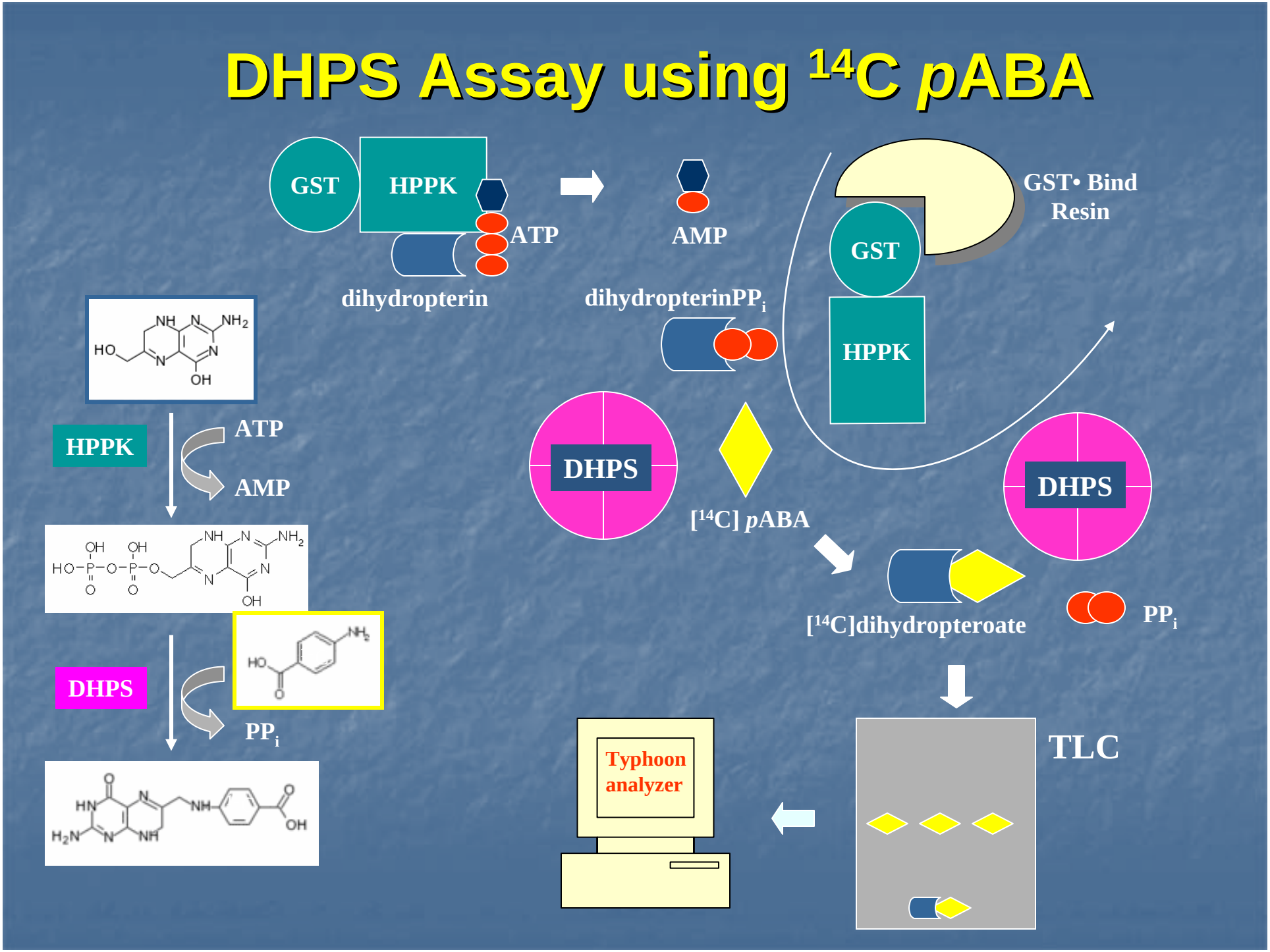
Docking Validation Study:
Enrichment at 1% for Various Docking Programs and Scoring Functions



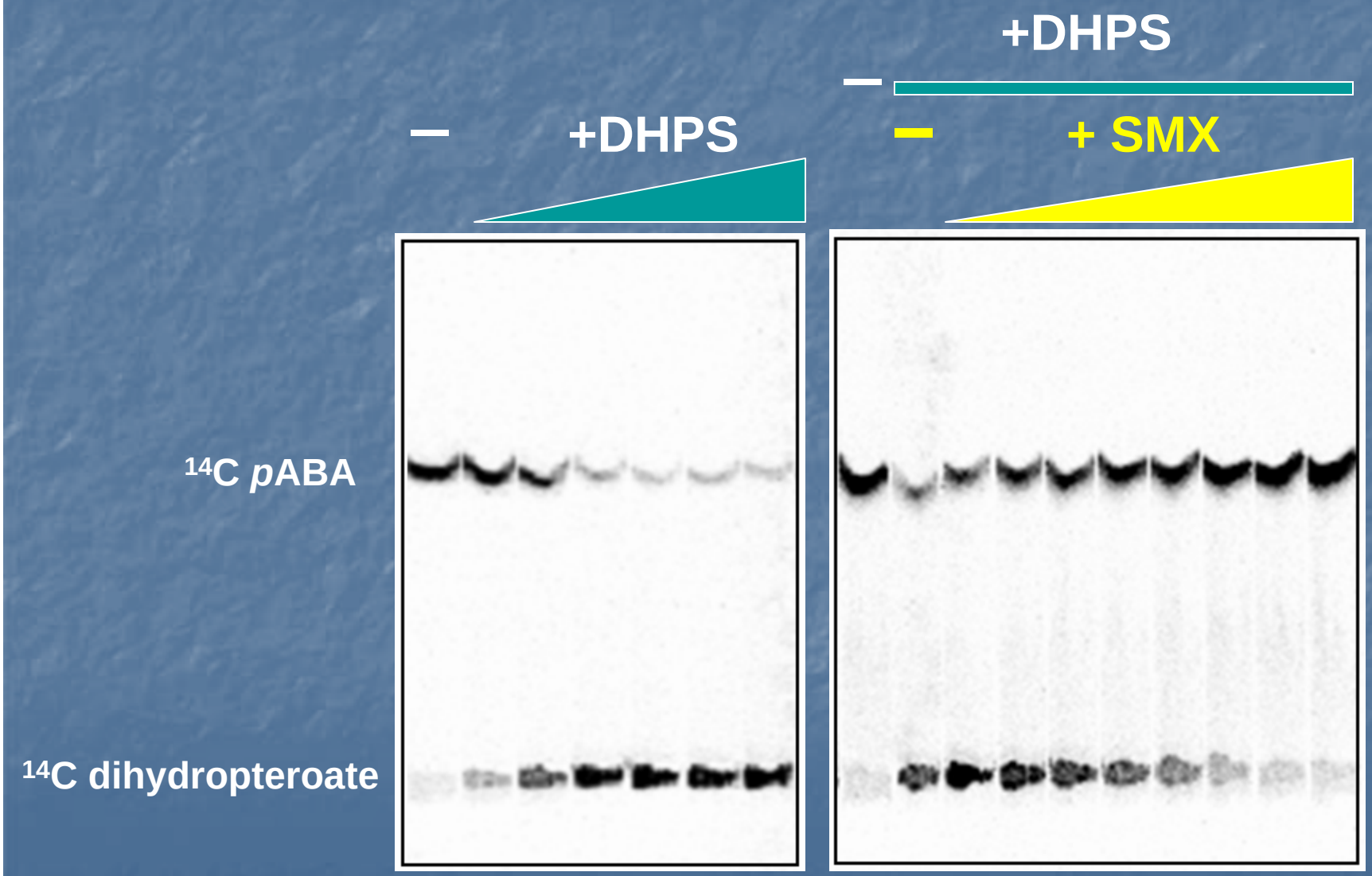
Glide Docking: Enrichment Results



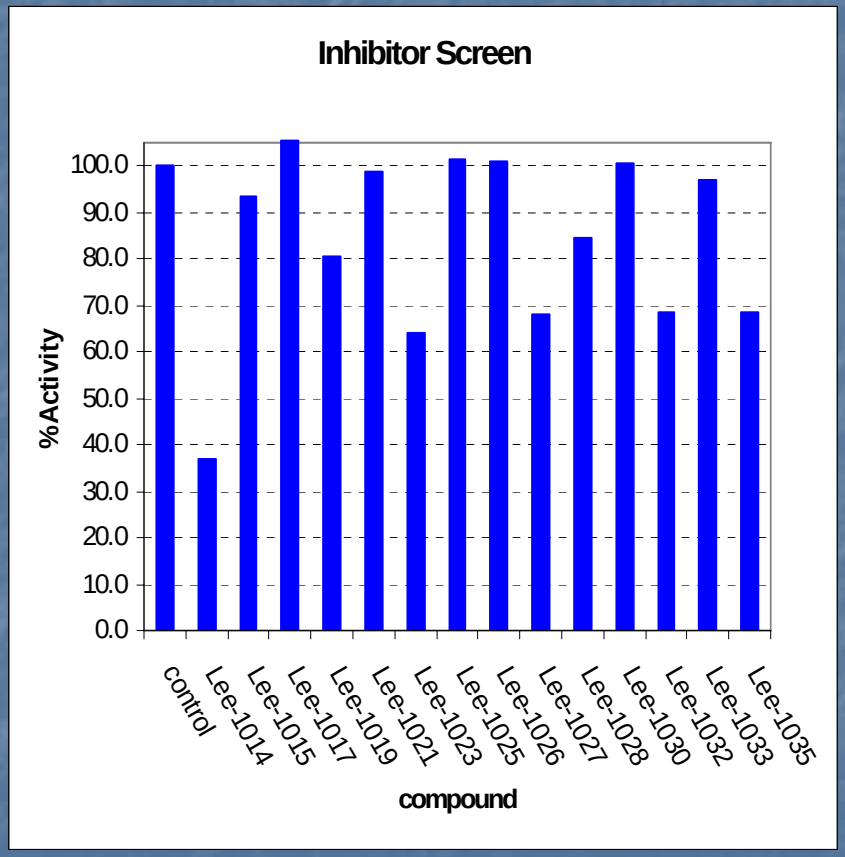
DHPS Assay using ^{14}C *p*ABA



DHPS Assay: Without and With SMX



DHPS Assay of some of the Virtual Screening Hits



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